[Revised manuscript of JBC: M8:07764]

Supplementary Information

Sunhong Kim, Yongsung Kim, Jiwoon Lee & Jongkyeong Chung

This section contains:

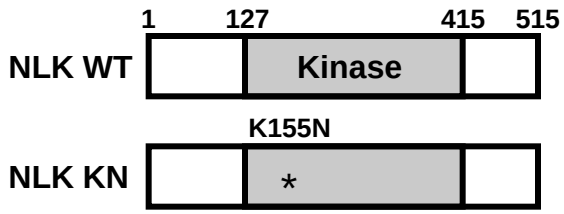
1) Supplementary Experimental Procedures
2) Supplementary Figures (1-8)
3) Supplementary Figure Legends (Supplementary Figures 1-8)

1) Supplementary Experimental Procedures

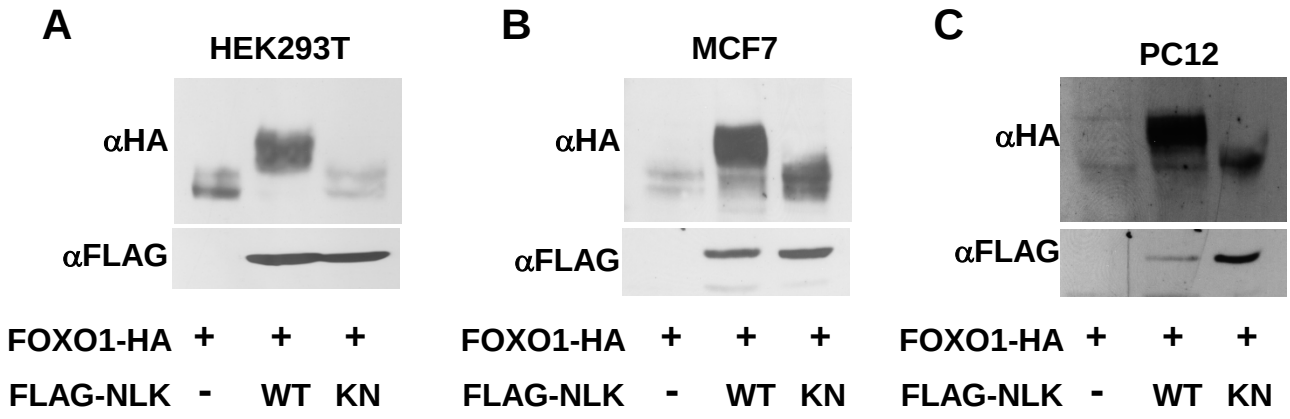
Drosophila strains – nemo (*Drosophila NLK*) loss of function mutant (nmo^{P1}/$nmo^{j147\text{-}1}$) fly strain was kindly provided by Dr. K.W. Choi (KAIST, Korea).

Quantitative real time PCR primers - The primer sequences used for PCR were as followings: *dInR*-specific primers (5'-GGAGAGCCCCAAAAGCCGAGC-3' for 5' and 5'-GCGCGTTAGCACGCCATAAGGC-3' for 3'), *Thor*/*d4E-BP*-specific primers (5'-CTCCTCGACGCCCGGCG-3' for 5' and 5'-GGGCACGTTGGACGGCGGAG-3' for 3'), The expression level from each sample was normalized to the mRNA expression level of a housekeeping gene, ribosomal protein 49 (*rp49*).

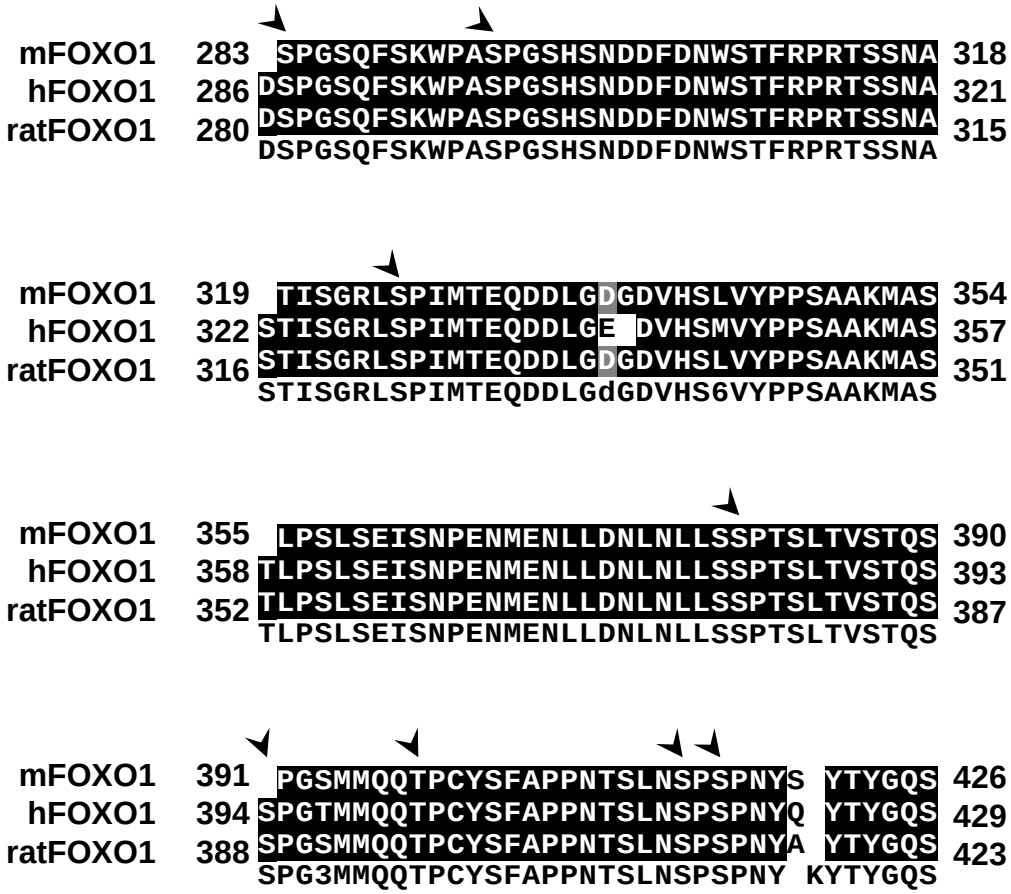
2) Supplementary Figures 1-8



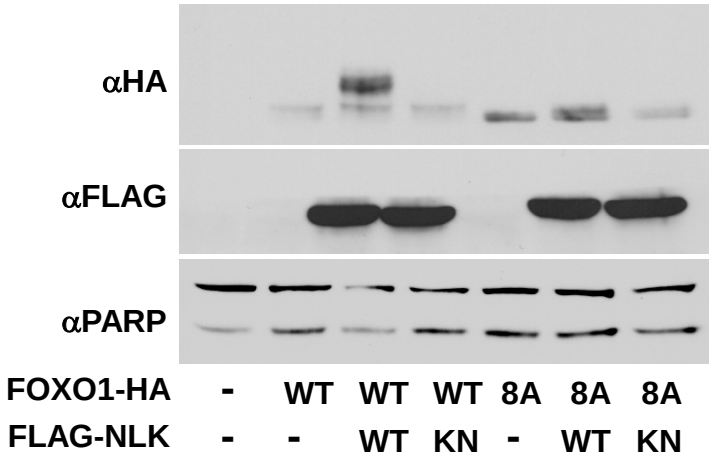
Supplementary Figure 1.



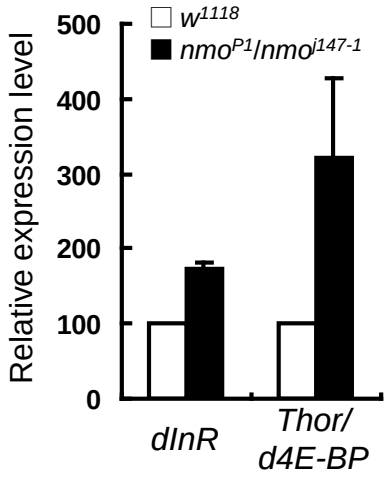
Supplementary Figure 2.



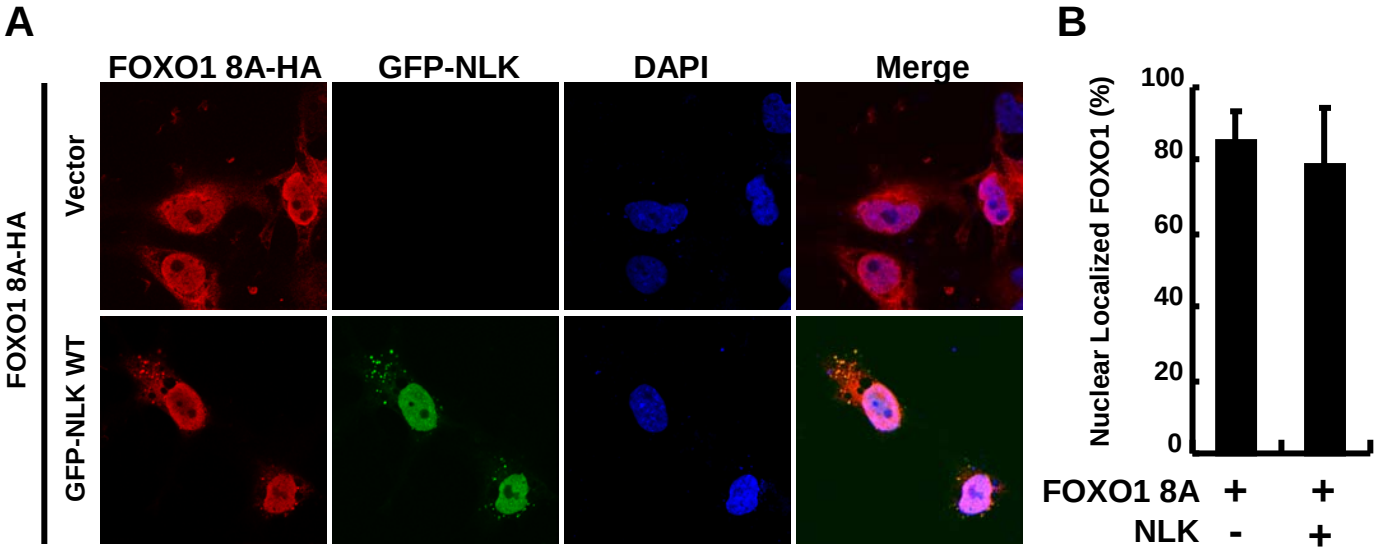
Supplementary Figure 3.



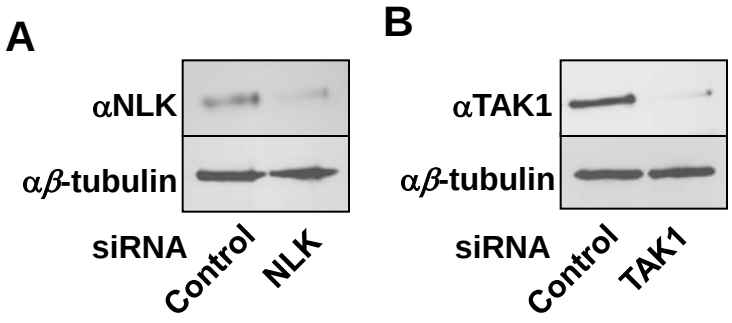
Supplementary Figure 4.



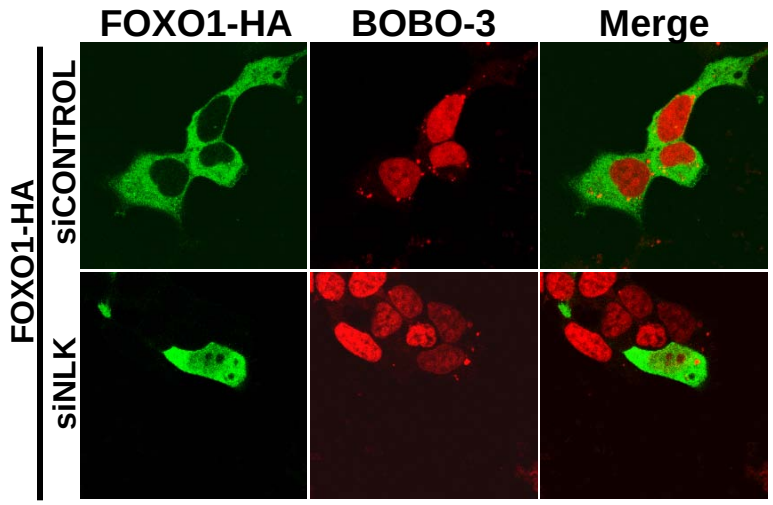
Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure 7.



Supplementary Figure 8.

3) Supplementary Figure Legends (Supplementary Figures 1-8)

Supplementary Figure 1. A diagram of NLK wild type (WT) and kinase-inactive (KN) mutant used in experiments. Numbers refer to the amino acid positions of human NLK.

Supplementary Figure 2. NLK phosphorylates FOXO1 in various cell lines. FOXO-HA WT and FLAG-NLK WT or KN constructs were co-transfected in HEK293T **(A)**, MCF7 **(B)**, and PC12 **(C)** as indicated. After 36 hours of transfection, the cells were lysed for immunoblot analyses with anti-HA and anti-FLAG antibodies.

Supplementary Figure 3. Potential phosphorylation sites of FOXO1 by NLK. Amino acid sequences in C1-region of human, mouse and rat FOXO1 were aligned. Ser284, Ser295, Ser326, Ser380, Ser391, Thr399, Ser413, and Ser415 of mouse FOXO1 are highly likely to be the possible phosphorylation sites by NLK. Numbers refer to the amino acid positions.

Supplementary Figure 4. NLK inhibits apoptosis through the FOXO1 phosphorylation. COS1 cells were transiently transfected with FOXO1-HA WT or 8A and NLK WT or KN constructs as indicated. After 36 hours of transfection, the cells lysates were subjected to immunoblot with anti-HA, anti-FLAG and anti-PARP antibodies.

Supplementary Figure 5. The transcription levels of *dInR* and *Thor/d4E-BP* in w^{1118} and *nemo* null (nmo^{P1}/nmo^{j147-1}) flies were analysed by qRT-PCR. Ribosomal protein 49 (*rp49*) was used as internal control; N=3. Bars indicated mane ±SD.

Supplementary Figure 6. FOXO1 8A mutant is localized in nucleus regardless of NLK. **(A)** COS1 cells were transiently transfected with FOXO1 8A-HA alone, FOXO1 8A-HA and GFP-NLK WT constructs. After 24 hours of transfection, the cells were fixed and subjected to immunocytochemistry. Then, the cells were stained with DAPI (nucleus, blue) and anti-HA (FOXO1 8A, red) antibody. GFP-NLK WT was depicted in green. **(B)** Quantification of COS1 cells with nuclear localized FOXO1 8A shown in **(A)**. FOXO1 8A-HA alone, N=56; FOXO1 8A-HA and GFP-NLK WT, N=67. Bars indicate mean ±SD.

Supplementary Figure 7. . Verification of siRNA-mediated knockdown of NLK and TAK1 at the protein level. **(A** and **B)** Control, *NLK* **(A)** or *TAK1* **(B)** siRNA-treated HEK293T cells were lysed, then the lysates were subjected to immunoblot with anti-NLK, -TAK1 and -β-tubulin antibodies.

Supplementary Figure 8. NLK regulates the localization of FOXO1. HEK293T cells were transfected with siRNA of *NLK* and FOXO1-HA WT construct. After 72 hours of transfection, cells were stained with BOBO-3 (nucleus, red) and anti-HA antibody. FOXO1 was depicted in green. The images shown are representative of three independent experiments.